

Shedding of Ovine Herpesvirus 2 in Sheep Nasal Secretions: the Predominant Mode for Transmission

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Ovine herpesvirus 2 (OvHV-2), the major causative agent of malignant catarrhal fever in ruminant species worldwide, has never been propagated in vitro. Using real-time PCR, a striking, short-lived, peak of viral DNA, ranging from 10⁵ to over 10⁸ copies/2 µg of DNA, was detected in nasal secretions from over 60.7% of adolescent sheep (n = 56) at some point during the period from 6 to 9 months of age. In contrast, only about 18% of adult sheep (n = 33) experienced a shedding episode during the study period. The general pattern of the appearance of viral DNA in nasal secretions was a dramatic rise and subsequent fall within 24 to 36 h, implying a single cycle of viral replication. These episodes occurred sporadically and infrequently, but over the 3-month period most of the 56 lambs (33, or 60.7%) experienced at least one episode. No corresponding fluctuations in DNA levels were found in either peripheral blood leukocytes or plasma. In a DNase protection assay, complete, enveloped OvHV-2 virions were demonstrated in the nasal secretions of all sheep examined during the time when they were experiencing an intense shedding episode. OvHV-2 infectivity in nasal secretions was also demonstrated by aerosolization of the secretions into OvHV-2-negative sheep. The data herein show that nasal shedding is the major mode of OvHV-2 transmission among domestic sheep and that adolescents represent the highest risk group for transmission.

Malignant catarrhal fever (MCF) is emerging as a significant source of economic loss in several ruminant species, such as cattle, deer and, in particular, confined bison (4, 20, 21). Worldwide, most cases of MCF are caused by ovine herpesvirus 2 (OvHV-2), which exists as a ubiquitous subclinical infection in domestic sheep. Research progress historically has been constrained by the inability to propagate OvHV-2 in vitro or to readily reproduce the disease experimentally and by a lack of reliable detection methods for the virus. Newer technologies, such as competitive enzyme-linked immunosorbent assay (cELISA) and PCR (1, 12, 13) and quantitative PCR (6, 8), have dramatically accelerated the understanding of the epidemiology of OvHV-2 in recent years, in both clinically susceptible species and in its own natural host, the domestic sheep. A number of studies have confirmed that under natural flock conditions the majority of lambs are not infected until after 2 months of age (16), which significantly differs from alcelaphine herpesvirus 1 infection in wildebeest calves (19, 20). Unlike wildebeest, placental transmission only rarely occurs in sheep (16). Colostrum and milk from infected dams have little role in viral transmission, even though they contain virus-infected cells (14, 16). Both lambs and adult sheep are susceptible to infection via horizontal short-distance transmission (15, 17). Passively acquired maternal immunity has been shown to not affect the rate of infection, which appears to be simply dose dependent (15). Although OvHV-2 DNA can be detected continuously in nasal secretions of most infected sheep, the high-

est levels of viral DNA predominantly occur between 6 and 9 months of age (10), suggesting this adolescent period as the time when most virus is shed into the environment.

Like other herpesviruses, OvHV-2 establishes lifelong persistent infection in its natural hosts through latency (20). Without causing clinically significant damage to its carrier host, the virus reactivates from the latent to the lytic mode, during which infectious virus is produced and transmitted to maintain viral reservoirs and to induce disease in other species. Lymphocytes are host cells for OvHV-2 in sheep, although which subsets are infected has not been clearly defined (2, 10). Little is known about the nature of the virus-carrier host relationship, such as the sites of productive viral infection. A source of cell-free, infectious virus is badly needed for studies of pathogenesis and immunological control. This study was designed to define OvHV-2 shedding patterns of infectivity in nasal secretions of adolescent sheep, as an early step toward the goal of identifying the sites of productive replication in vivo.

MATERIALS AND METHODS

Animals, sample collection, and preparation. Between 2001 and 2004, eight groups of sheep were used to define OvHV-2 shedding patterns. They consisted of adolescents (herein defined as sheep 6 to 9 months old) and adults (over 1 year old). All sheep were obtained from the U.S. Sheep Experiment Station, Dubois, Idaho, except for groups 2 and 8. The 10 sheep in group 2 were obtained from a private flock at Connell, Wash. Group 8 consisted of seven adult sheep, four of which were from an OvHV-2-negative sheep flock maintained in Pullman and subsequently infected with OvHV-2 by experimental aerosol transmission in 2003. The other three were left from group 6 (as “high shedders,” defined as having at least two shedding peaks with over 10⁶ viral DNA copies/2 µg of DNA in their nasal secretions). The details about numbers of animals, ages, sampling period, and total nasal sample days in each group are summarized in Table 1. All experiments, except those involving groups 3 and 4, were conducted at the Washington State University animal facilities in Pullman, Wash. Experiments

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TABLE 1. Summary of animal groups and sample collection dates

Group ^a	Source	No. of animals	Sex ^c	Age ^b (median)	Sampling period	Total sample days
1 (adolescent)	Dubois, Idaho	15	F	150–185 days (172)	Oct. 10, 2001–Dec. 20, 2001	341 ^d
2 (adolescent)	Cornell, Wa.	10	F/M	~180 days	Dec. 23, 2001–Feb. 22, 2002	566 ^e
3 (adult)	Dubois, Idaho	10	F	24–48 mos	Mar. 28, 2002–Jun. 5, 2002	697 ^f
4 (adult)	Dubois, Idaho	10	F	24–48 mos	Mar. 28, 2002–Jun. 5, 2002	700
5 (adolescent)	Dubois, Idaho	16	F	178–192 days (188)	Oct. 11, 2002–Dec. 19, 2002	1003 ^g
6 (adult)	Pullman, Wa.	6	F/M	16–18 mos	Oct. 11, 2002–Dec. 19, 2002	420
7 (adolescent)	Dubois, Idaho	15	M	182–187 days (187)	Oct. 8, 2003–Jan. 9, 2004	1152 ^h
8 (adult)	Pullman, Wa. ⁱ	7	F/M	16–18 mos	Oct. 8, 2003–Jan. 9, 2004	537 ⁱ

^a An adolescent sheep was defined as about 6 to 9 months of age; an adult was defined as over 1 year of age. All animals were naturally infected, except four animals in group 8 that were experimentally infected.

^b Age at the first sampling date.

^c All males (M) were wethers in this study; all females were nonpregnant except 10 sheep in group 4.

^d Four animals were terminated early for unrelated reasons.

^e Two animals had early terminations.

^f One animal died 3 days before the termination of the experiment.

^g Two animals in group 5 were terminated early.

^h In group 7, two animals were terminated early and no samples were collected on weekends.

ⁱ In group 8, one animal was terminated early and no samples were collected on weekends.

^j Four sheep in the group were experimentally infected with OvHV-2 by aerosol transmission, and three sheep were selected as defined high shedders from the previous year (group 5).

with groups 3 and 4 were carried out at the U.S. Sheep Experiment Station at Dubois, Idaho. Groups 3 and 4 consisted of 20 age-matched infected ewes, 10 of which were pregnant and 10 of which were nonpregnant. Nasal secretion samples were collected daily from all groups of animals except group 1, from which the samples were collected at 3-day intervals for the first 40 days and daily thereafter. EDTA blood samples were collected weekly from all animals in groups 1 and 2 and daily from all animals in groups 5 and 6. Body temperatures were monitored daily from each individual sheep in groups 5 and 6. No blood samples were taken from the animals in groups 3, 4, 7, and 8 during the study period, except for a single blood sampling 2 weeks before the nasal sampling began, to confirm the OvHV-2 infection status in these individuals. For the aerosol transmission experiment, two 9-month-old uninfected sheep that had been raised in an isolation facility at Washington State University were used.

Additional samples, including ocular secretions, feces, and urine, were collected from six sheep experiencing a shedding episode. Twelve sets of placental tissues and amniotic fluid were collected from OvHV-2-positive lambing ewes from a local sheep flock near Moscow, Idaho.

CELISA, seminested PCR, and real-time PCR. Plasma samples were collected for MCF viral antibody detection by a cELISA. The protocol for the cELISA was described previously (11). Animals were defined as infected or noninfected by PCR detection of OvHV-2 DNA in peripheral blood leukocytes (PBLs). For DNA preparation, PBLs were collected from EDTA blood, red blood cells were lysed with Tris-HCl buffer containing 0.87% ammonium chloride, and PBLs were washed with phosphate-buffered saline (PBS). DNA was purified using the Fast DNA kit (Q-Bio Gene, Carlsbad, Calif.). DNA from nasal secretion samples was also purified by the Fast DNA Kit. Purified DNA was quantitated by spectrophotometry at 260 nm. The procedure for OvHV-2-specific seminested PCR was as described previously (13) with two modifications: (i) 0.1 to 0.5 µg of DNA was used in the primary reaction mixture, and (ii) 2 µl of PCR products from the primary reaction was used in the secondary reaction mixture. OvHV-2 real-time PCR developed by Hussy et al. (8) was used with a few modifications (9). Briefly, the 25-µl PCR mixture for one reaction contained 23 µl of TaqMan Universal Master Mix (Perkin-Elmer Corporate, Wellesley, Mass.), 240 nM forward primer (oF-OvHV-2, 5'-TGGTAGGAGCAGGCTACCGT-3'), 600 nM reverse primer (oR-OvHV-2, 5'-ATCATGCTGACCCCTGCAG-3'), 80 nM probe (oP-OvHV-2, 5'-TCCACGCCGTCCGCACTGTAAGA-3'), and 2 µl of diluted template DNA (0.1 to 0.5 µg) or standard DNA. The standard DNA was a plasmid cloned in a previous study, which contained a 309-bp fragment of OvHV-2 DNA (6). The template DNA was diluted in water, and the standard DNA was diluted in a solution containing 100 µg of herring sperm DNA/ml. The thermal cycle profile used was as follows: 95°C for 10 min to denature DNA and activate the AmpliTaq Gold DNA polymerase, followed by 40 cycles at 60°C for 1 min and at 95°C for 15 s. Amplifications were performed in an iCycler iQ real-time PCR detection system (Bio-Rad Laboratories, Hercules, Calif.), and data were analyzed using software (version 2.3) provided by the manufacturer.

DNase protection assay and aerosol transmission experiment. The method for preparation of nasal secretion samples for the DNase protection experiment was

described in detail previously (9). Briefly, nasal secretion samples were diluted with PBS and centrifuged to remove cellular debris. Virions from the supernatant were sedimented by centrifugation for 2 h at 41,270 × *g* at 4°C. The pellets were treated with various combinations of NP-40, pronase, and DNase, and the surviving DNA was purified for PCR. Positive PCR signals from the sample treated with both pronase and DNase indicated the presence of complete, enveloped OvHV-2 virions in that nasal secretion sample (9).

In the aerosol transmission experiment, two uninfected 9-month-old sheep were aerosolized with nasal secretions containing over 10⁵ OvHV-2 DNA copies/2 µg of total DNA as detected by real-time PCR. To prepare inoculum for transmission experiments, eight nasal swabs were collected from an individual sheep in group 2 that was experiencing an intensive shedding episode. The swabs were placed in a tube containing 6.0 ml of cold PBS and agitated gently, and excess fluid was expressed. The supernatant was collected after clarification by low-speed centrifugation at 129 × *g* for 5 min. Each uninfected recipient sheep received 2 ml of inoculum (containing about 7.5 × 10⁸ viral DNA copies) by aerosolization. For aerosolization, a liter-sized plastic bottle with the bottom removed was used to hold the sheep nose to concentrate the aerosol emitted by the device. The inoculum was delivered by a commercial nebulizer (Devilbiss Pulmo-Aide, Sunrise Medical, Somerset, Pa.). Blood and nasal secretion samples were collected weekly from each animal for antibody and viral DNA assays.

Statistical analysis. The data for the shedding frequencies were analyzed with Epi Info 6 version 6.04 (Centers for Disease Control and Prevention and World Health Organization [Geneva, Switzerland]). The data for shedding intensities were analyzed by using an unpaired *t* test with Welch correction (GraphPad Software, Inc., San Diego, Calif.). Statistical significance was determined as a *P* value of <0.05.

RESULTS

Shedding frequency in adolescents and adult sheep. As used herein, a shedding peak day is arbitrarily defined as the day on which an individual animal had at least 10⁵ OvHV-2 genome copies/2 µg of DNA in its nasal secretions. As shown in Table 2, the 15 adolescent sheep in group 1 experienced 10 shedding peak days, which corresponded to 34.1 sheep-days per shedding event. Copy numbers at the peak ranged from 118,000 to 6,350,000 copies/2 µg of DNA (2,004,905 ± 764,281 [standard error of the mean]; *n* = 10). Only 7 out of 15 sheep experienced shedding events, two of which had two and three events, respectively, during the study period. In group 2, six shedding events (93.3 sheep-days/shedding event) were detected among 10 adolescent sheep, for a total of 566 sheep-days. The shedding intensities ranged from 147,000 to 2,413,000 copies/2 µg

TABLE 2. OvHV-2 shedding events in adolescent and adult sheep

Group, age	No. of animals	Study period (days)	Total sheep-days	Total shedding peak days ^a	Days per shedding event ^b
1, adolescent	15	66	341	10	34.1
2, adolescent	10	62	566	6	93.3
3, adult	10	70	697	0	>697
4, adult	10	70	700	0	>700
5, adolescent	16	70	1,003	23	43.6
6, adult	6	70	420	1	420
7, adolescent	15	94	1,152	19	60.6
8, adult	7	94	537	5	107.4
Total, adolescent	56	~73	3,062	58	52.8
Total, adult	33	~76	2,354	6	392.3

^a A shedding peak day was arbitrarily defined as a day on which an individual animal had at least 10^5 OvHV-2 genome copies/2 μ g of DNA in its nasal secretions.

^b Days per shedding event equals total sheep days divided by total shedding peak days.

of DNA ($1,242,075 \pm 507,075$; $n = 6$). Five animals experienced shedding events in this group, one of which experienced two events. In group 5, a total of 23 shedding peak days were observed among 16 adolescent sheep. The sample set from this group comprised 1,003 sheep-day samples, which corresponded to 43.6 sheep-days/shedding event. The viral DNA levels in this group ranged from 114,000 to 232,500,000 copies/2 μ g of total nasal DNA ($24,858,000 \pm 11,546,123$; $n = 23$). Twelve out of the 16 sheep in this group experienced at least one shedding peak during the study period. Four out of these 12 animals experienced multiple shedding peaks, which ranged from two to four peaks. In group 7, a total of 19 shedding peak-days were detected in 1,152 sheep-day samples from the 15 adolescents, equal to 60.6 sheep-days/shedding event. OvHV-2 copy numbers from sheep in this group ranged from 104,000 to 21,900,000 copies/2 μ g of total nasal DNA ($2,597,000 \pm 1,249,656$; $n = 19$). A total of 10 out of the 15 experienced at least one shedding episode, and 2 of these had two to three peak days within 72 h. There was no statistically significant difference in the frequency of OvHV-2 shedding among the adolescent sheep groups ($P > 0.05$). The overall shedding frequency among adolescent sheep was 52.8 sheep-days per shedding event (58 events in 3,062 sheep-days).

In the adult groups, a single shedding event (550,000 copies/2 μ g of DNA) was detected in a ewe from group 6. No shedding events were observed in either the pregnant ewes (group 3) or the nonpregnant ewes (group 4) during the 10-week study period. However, in group 8, a total of five shedding peaks were identified from seven ewes (537 sheep-days). The viral DNA levels in this group ranged from 198,000 to 6,019,000 copies/2 μ g of DNA ($1,803,000 \pm 1,108,820$; $n = 5$). The shedding frequency in this group equaled 107.4 sheep-days/shedding event. The overall frequency among adult ewes from all groups combined was 392.3 sheep-days per shedding event (six events in 2,354 sheep-days). Although no significant difference in shedding frequency was observed between groups of adult ewes in groups 3, 4, and 6 ($P > 0.05$) or between group 6 and group 8 ($P > 0.05$), a significant difference was observed between group 8 and groups 3 and 4 ($P < 0.05$). When adult sheep (group 8) were compared with the adolescent sheep group, no significant difference was seen in the shedding peak frequency ($P > 0.05$). However, a significant difference was

apparent ($P < 0.000001$) when total numbers of adolescents and adult sheep were compared. Adolescent sheep were 7.43 times (95% confidence interval, 3.31 to 17.17; $P < 0.000001$) more likely to shed the virus through nasal secretions than the adult sheep in this study.

Shedding intensity in adolescent and adult sheep. In those animals with detectable viral DNA in nasal secretions, maximum OvHV-2 copy numbers ranged from 163 to 232,500,000 copies/2 μ g of total DNA (Fig. 1A) ($13,889,000 \pm 5,997,074$; $n = 56$) in adolescent sheep, compared to 64 to 6,019,000 copies/2 μ g of total DNA (Fig. 1B) ($288,100 \pm 190,388$; $n = 33$) in adult sheep. The peak mean OvHV-2 copy number in nasal secretions from adolescent sheep was 48.2 times greater than that in adults ($P < 0.05$). Considering all nasal samples in the study, the mean viral DNA level in total nasal secretion samples from adolescent sheep ($262,189 \pm 113,817$; $n = 3,062$) was 61 times greater than that in total nasal secretion samples from adult sheep ($4,278 \pm 2,721$; $n = 2364$) ($P < 0.05$).

OvHV-2 shedding patterns in sheep. A total of 58 shedding peaks were observed from 3,062 sheep-days in 56 adolescent sheep during a period of about 3 months. Forty-one out of 56 (73%) individual adolescents had at least one shedding episode ($\geq 100,000$ copies) during the study period. Based primarily on duration, as shown in Fig. 2 the shedding episodes were divided into three categories: only one peak, two distinct peaks, or multiple peaks. Thirty-two adolescent sheep (78%) had either one peak or two distinct peaks, each of which lasted less than 24 h (Fig. 2A and B). Only nine adolescent sheep (22%) had shedding episodes that lasted 48 h or longer (Fig. 2C). No significant fluctuations in body temperature were observed in relationship to the shedding episodes.

Detection of enveloped OvHV-2 virions and viral infectivity in nasal secretions. A total of 10 nasal secretion samples from sheep experiencing a shedding episode were assayed for the presence of complete, enveloped OvHV-2 virions. All 10 samples tested positive by PCR after treatment with pronase and DNase (data not shown). In order to demonstrate infectivity, two OvHV-2-negative sheep were aerosolized with 1 of the 10 nasal secretion samples collected for the above DNase protection assay. Both sheep seroconverted and became PCR positive at 2 and 3 weeks, respectively, after nebulization (Fig. 3).

Levels of OvHV-2 DNA in other samples. As shown in Table 3, levels of OvHV-2 DNA did not rise in concert with the levels in nasal secretions during shedding episodes in any other samples that were examined from five sheep, which included PBLs, plasma, urine, feces, and ocular and oral fluids, with the exception of one oral sample. The daily collected blood samples from the three sheep that experienced at least one shedding episode during the 10-week study period revealed that OvHV-2 DNA levels in PBLs remained low, from a few copies to about 2,000 copies/2 μ g of DNA (mean, 135; $n = 210$). There was no correlation between OvHV-2 DNA levels in nasal secretions and the levels in PBLs or plasma of individual sheep (data not shown).

Low levels of OvHV-2 DNA in placentas and amniotic fluid. In order to determine whether placental tissues and amniotic fluid were significant sources of the virus for transmission, 12 sets of placental and amniotic fluid samples were collected from OvHV-2-infected lambing ewes in a private farm flock. No information was available on their nasal secretion copy

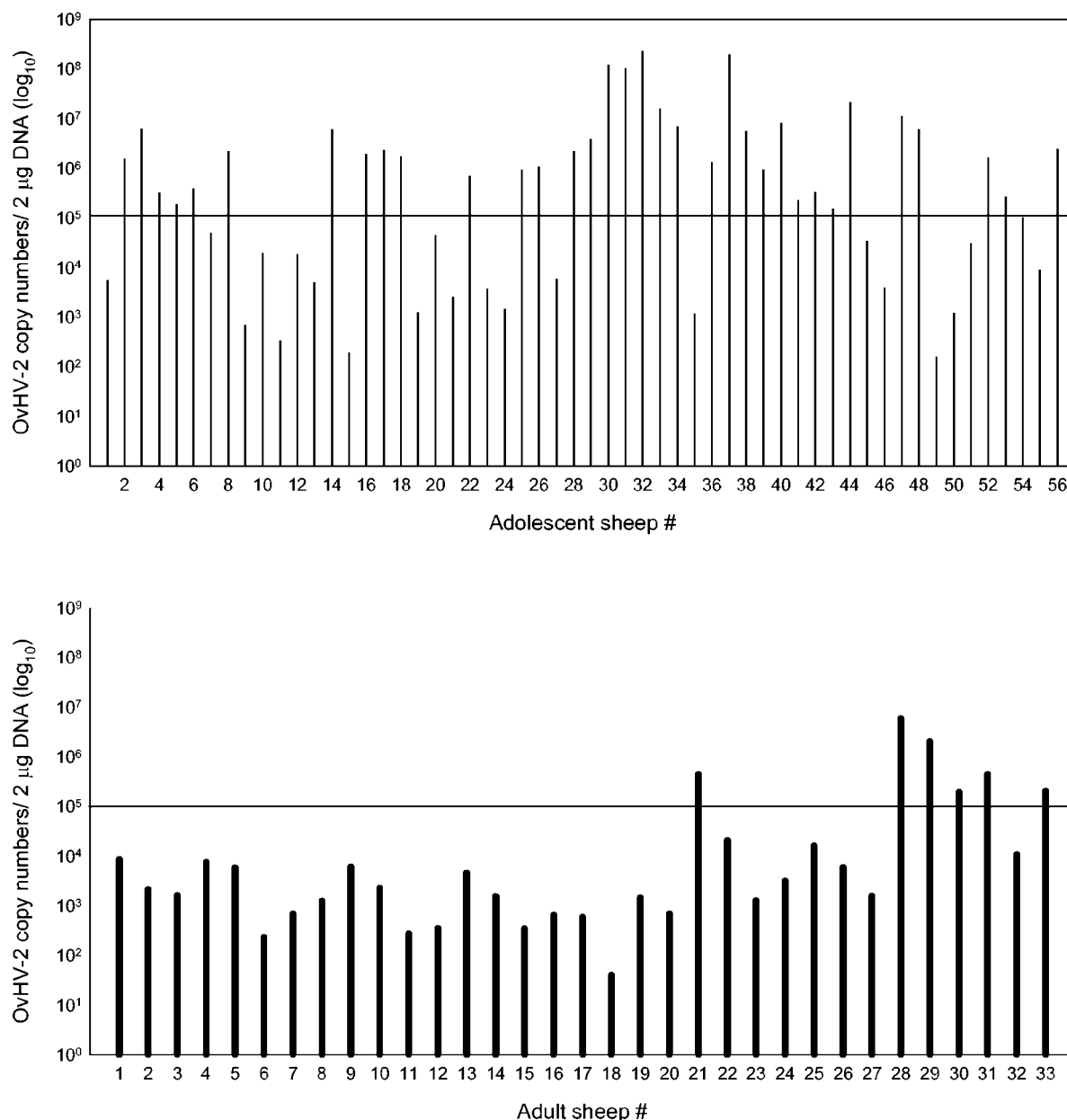


FIG. 1. Highest levels of OvHV-2 DNA recorded in nasal secretions of adolescent and of adult sheep. (A) Results for 56 adolescent sheep (groups 1, 2, 5, and 7) beginning at about 6 months and ending at about 9 months of age. (B) Results for 33 adult sheep samples taken over a 3-month period. Among these were 10 lambing ewes (group 2) and 23 nonpregnant ewes (group 4, nos. 11 to 20; group 5, nos. 21 to 26; group 8, nos. 27 to 33). The horizontal lines at 10^5 viral DNA copies arbitrarily define a minimum shedding peak day for individual animals.

numbers. Only very low levels (<100 copies/2 µg of DNA) of OvHV-2 DNA were detected in the placental tissues and amniotic fluids. No enveloped OvHV-2 virions were detected in any of 12 amniotic samples examined in the DNase protection assay (data not shown).

DISCUSSION

This study provides strong evidence that the predominant route by which OvHV-2 is shed from its major carrier host, the sheep, is via nasal secretions. Moreover, this shedding occurs in a manner that displays unique shedding kinetics and an age-related frequency. The data established that nasal secretions

from shedding sheep contain (i) a high level of OvHV-2 DNA, (ii) complete enveloped OvHV-2 virions, and (iii) viral infectivity. A previous study from this laboratory showed high levels of OvHV-2 DNA in nasal samples (10). However, since viral DNA can exist in latently infected cells from nasal secretions or other tissues, detection of viral DNA per se by PCR does not constitute proof of viral replication or shedding. However, sufficiently sharp and abrupt fluctuations in viral DNA content of secretions suggest that viral replication might be occurring at that site. Although it is not yet known precisely what level of viral DNA is indicative of active replication, all nasal secretion samples that contained $\geq 10^5$ copies per 2 µg of total DNA

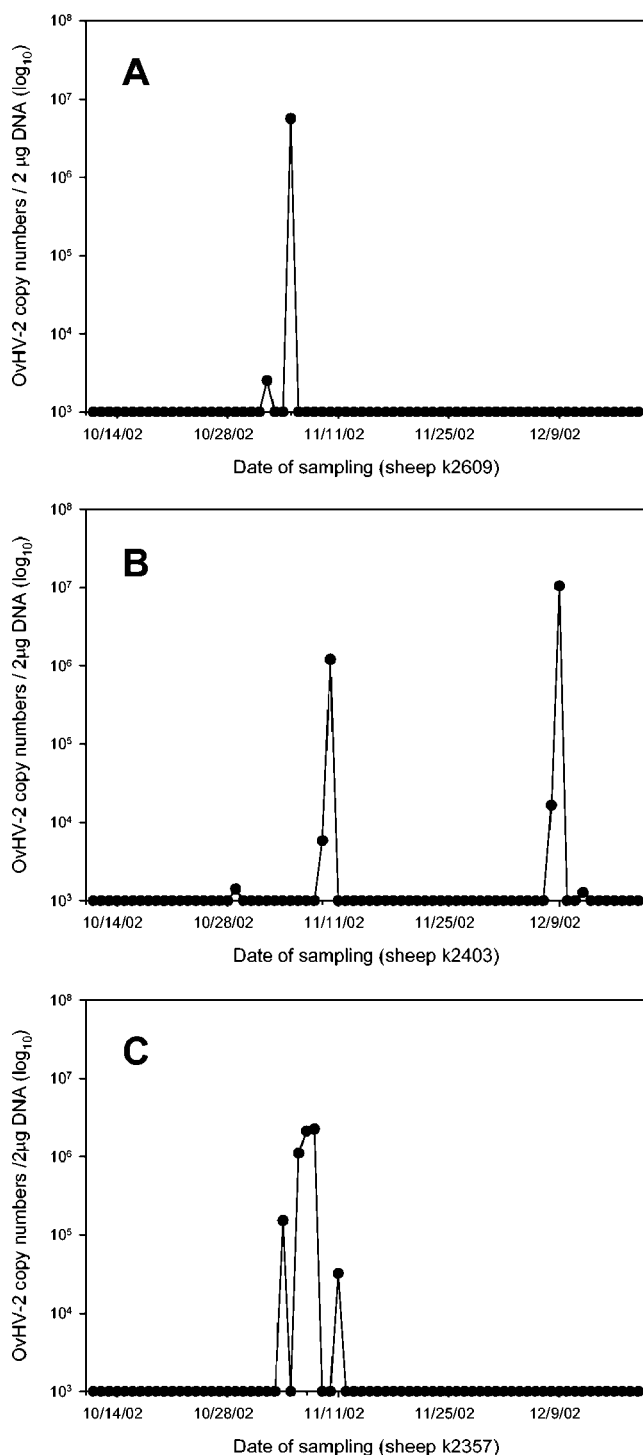


FIG. 2. Real-time PCR depicting OvHV-2 shedding episodes from three individual adolescent sheep (6 and 9 months of age). (A) Single peak lasting less than 24 h (K2069); (B) two distinct peaks, both lasting less than 24 h (K2403); (C) multiple, almost overlapping peaks, with a total duration of about 96 h (K2357).

proved in a previous study (9) to contain complete, enveloped OvHV-2 virions when examined in a DNase protection assay. Additionally, infection of OvHV-2-negative sheep by aerosolization of the nasal secretions from sheep experiencing a shed-

ding episode has been achieved. Although only one experiment of such aerosol transmission is presented here, additional aerosol transmission experiments in sheep with varied doses of nasal secretion inocula have recently been completed (N. Taus et al., unpublished data).

Recently, Hussy et al. (7) demonstrated high levels of OvHV-2 DNA in the semen of rams and suggested an important role for sexual transmission. Data from the present study, however, are strongly indicative that the respiratory tract constitutes the primary transmission route, but they do not rule out a possible minor role for other routes of transmission. The fact that most lambs are infected before they reach sexual maturity (16) argues against a major role for sexual transmission in maintenance of the virus in sheep populations.

The majority of viral shedding originates from adolescent lambs, 6 to 9 months of age. About 73% of the adolescents in this study experienced at least one shedding episode during the 3-month study period. Both the frequency and the intensity of shedding episodes in adolescent sheep were significantly higher than in adult sheep. Sheep of this age represent the greater danger to clinically susceptible species. Several recent large MCF outbreaks have been linked to exposure to nearby congregations of adolescent sheep, either sheep feedlots or grazing flocks of lambs (T. B. Crawford et al., unpublished data). The reason that adolescents shed more virus than other age groups is unclear.

Under natural flock conditions, the majority of lambs acquire OvHV-2 at about 10 weeks of age. In general, viral DNA appears in PBLs before it appears in nasal secretions (10), suggesting that initial infection may occur in lymphocytes. We speculate that an adequate number of latently infected lymphocytes may be necessary to provide sufficient lymphocytes in the lytic replication mode in order to serve as a competent source of infectious virus to infect as-yet-unidentified permissive cells in the mucosa of the respiratory tract. It is unknown whether cell types other than lymphocytes in the respiratory tract can be latently infected. If so, the reactivation of these other latently infected cells may result in productive infection in the unidentified permissive cells in the mucosa of the respiratory tract. The control mechanism(s) responsible for the decline in shedding frequency and intensity after about 10 months of age is also not understood but may be involved in development of immunological responses with age and/or following adequate antigenic exposure.

Interestingly, there was no statistically significant difference in shedding frequency between adolescents and seven adult sheep in group 8. It is not completely clear why this particular group of adult sheep had a higher shedding frequency than other adult sheep groups in this study. However, it is worthy to note that these sheep in group 8 were derived from different backgrounds compared to other adult sheep. Four sheep in the group were experimentally infected with OvHV-2 by aerosol transmission, and the length of infection in these sheep was significantly shorter (only about 10 months) than in adult sheep in other groups. The remaining three sheep in this group were selected as defined high shedders from the previous year. It is possible that certain sheep that have a specific genetic background shed the virus more frequently than others, even in the adult stage. Horizontal transmission of OvHV-2 between adult sheep has been documented (17); therefore, caution should

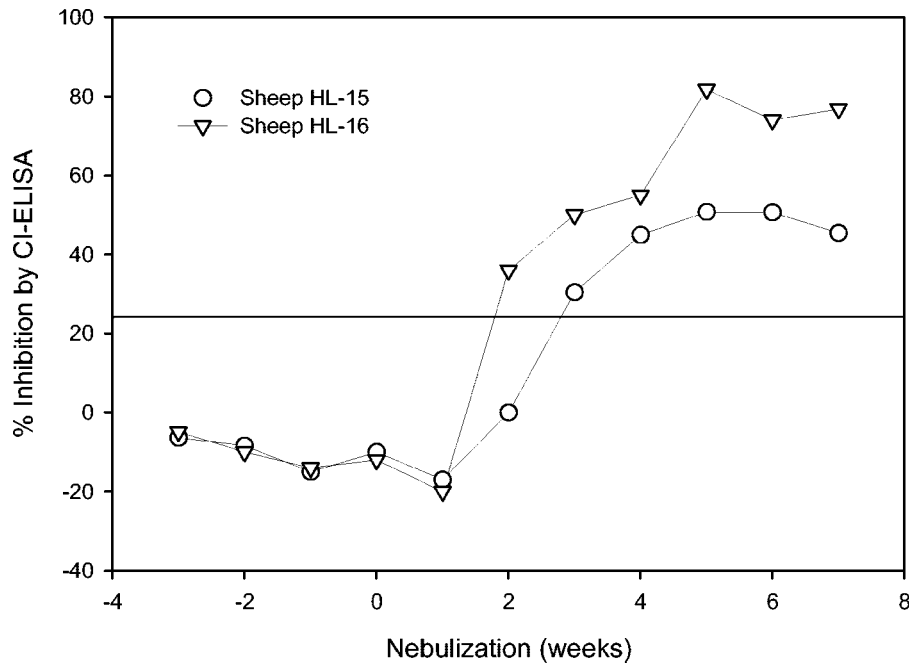


FIG. 3. Aerosolization experiment. Two OvHV-2-negative sheep received aerosols, each with 2 ml of nasal secretion samples obtained from sheep experiencing an intense shedding episode. Both sheep that received aerosols seroconverted at 2 or 3 weeks after aerosolization. The horizontal line represents the cutoff value at 25% inhibition.

always be taken for MCF losses in clinically susceptible species with direct or indirect exposure to adult sheep.

Surprisingly, the shedding episodes were predominantly in the form of sharp, intense but brief spikes, most of which began and declined again within a period of about 24 h. The best apparent explanation for this dramatic viral kinetic pattern is that it represents the product of a single cycle of viral replication in the mucosa of the respiratory tract. Two possible scenarios come readily to mind. The first is that the virus in the secretions stems from lytic replication in a large number of lymphocytes in the mucosa and submucosa of the respiratory tract, triggered by unknown host mechanisms. This seems unlikely, since no concurrent elevation of viral DNA was detected in PBLs or in plasma during shedding episodes. Another possibility is that OvHV-2 lytic replication occurs in epithelial cells, after infecting virus is released from a relatively smaller number of lymphocytes in their microenvironment. In this scenario, the epithelial cells are both susceptible and highly permissive, and they produce the sudden surge of infectious virus into the secretions. The fact that the shedding peaks are so transient suggests that the virus from the productively infected epithelial cells is not capable of reinfecting other epithelial cells; thus, the wave of replication is not propagated and ends quickly. Since we have established by experiments described herein that the virus in the secretions is infectious for other sheep, the likely candidate host cell for initial infection in the recipient sheep is the lymphocyte. It thus seems likely that in vivo OvHV-2 replication involves viral switching between two cell types, lymphocytes and epithelial cells, in a manner reminiscent of tropism switching recently reported for Epstein-Barr virus (3). If this is the case, it constitutes a fascinating mechanism whereby a persistent herpesvirus coexists with its natural host without excessive injury to the host. Because of the in-

ability of epithelial-origin virus to infect other epithelial cells, damage to the host is limited in scope. This phenomenon may also explain the fact this virus has never been propagated in vitro. If the tropism switch postulated herein does in fact exist, its mechanism, unknown at present, would be of considerable interest to virology.

Because a moderate increase in cases of sheep-associated MCF (SA-MCF) in cattle is often observed in the spring period (5, 18) and because the seasonality of wildebeest-associated MCF is apparently linked to wildebeest shedding during the calving season (19), lambs and lambing ewes have historically been considered to constitute the main source of sheep-associated (SA)-MCF virus. Shedding from ewes did not increase around the time of lambing, and it appears to bear no rela-

TABLE 3. OvHV-2 DNA copy numbers in nasal secretions and other samples from five sheep experiencing intense viral shedding events

Sheep no.	OvHV-2 copy number/2 µg of DNA by real-time PCR						
	Nasal ^a	PBL	Plasma	Ocular	Urine	Feces	Oral
367	674,000	332	— ^c	2,740	—	—	0 ^b
368	66,800	0 ^b	—	NT ^d	NT	NT	NT
464	59,454,000	4,745	—	6,750	NT	NT	642,867
375	905,000	1,200	—	455	—	—	254
366	75,000	467	—	0 ^b	—	—	181

^a All samples reported in this table were obtained from those sheep whose nasal secretions on preliminary screening earlier that day contained OvHV-2 DNA copy numbers exceeding 10^5 per 2 µg of DNA.

^b Samples were positive by OvHV-2 nested PCR, but with zero copies by real-time PCR.

^c Samples were negative by OvHV-2 nested PCR and had zero copies by real-time PCR.

^d NT, not tested.

tionship to parturition. The data herein, plus the facts that the majority of lambs are not infected before 2 to 3 months of age (16) and that most nasal secretions are PCR negative prior to 5 months of age, constitute strong evidence that neither reproductive tissues nor neonatal lambs are the source of transmissible virus (14, 16). A previous study from this lab failed to identify any seasonal pattern in the levels of viral DNA in nasal secretions of adult sheep (10). In order to confirm this earlier observation two groups of sheep, which included both lambing ewes and nonpregnant ewes, were sampled daily and examined for shedding. No difference in shedding frequency was observed between the two groups. Furthermore, significant levels of viral DNA were not found in any of the placental tissues or amniotic fluid collected from 12 OvHV-2-infected lambing ewes. Therefore, any higher rate of SA-MCF in the spring must be due to other factors, such as elevated levels of virus shed by adolescents during late winter, or ill-defined climatic or other environmental factors that influence virus survival and therefore the efficiency of transmission (10).

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